DRAFT

ENVIRONMENTAL EVALUATION PROCEDURES FOR WASTE MANAGEMENT AREAS AT ROCKY FLATS

PREPARED FOR

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I. INTRODUCTION

This manual is intended to serve as a guide for those preparing environmental evaluation work plans for solid waste management units (SWMUs) at the Rocky Flats Plant near Golden, Colorado. The primary purpose of an environmental evaluation is to characterize the physical and biological attributes of a site and to determine whether and to what extent human activities and/or contaminants are adversely impacting the local and adjacent environs of a SWMU. This determination is an essential prerequisite to decisions concerning the necessity of environmental remediation, and the extent of remedial action should it be warranted.

It is important to note that while relatively standardized methods exist to characterize terrestrial and aquatic sites, the task of determining whether adverse impacts are occurring, and what contaminants or practices are causing the impacts, is formidable. A basic problem is that most SWMUs have been impacted by more than one factor and the question of how multiple factors interact is seldom well-understood. Another problem is that the SWMUs were not characterized prior to the impact. This brings up the necessity of studying "reference" sites (i.e. those that are believed or shown to be unimpacted, yet ecologically similar to the sites suspected of being impacted). True "control" sites are not likely to be found because of the high natural ecological variability at Rocky Flats and the complex, poorly-documented land management history of the area. Finally, the SWMUs vary in size, shape and proximity to other SWMU's, thus adding many dimensions of complexity.

Despite these difficulties, environmental laws and interagency agreements require a practical evaluation of the SWMUs so that necessary decisions concerning remediation can be made. This manual was prepared under the

philosophy that environmental evaluations, if they are to be scientifically defensible, publicly credible, and useful to good management decisions, should be carried out with sound scientific methods, adequate quality control, and statistical rigor. It is the collective responsibility of the Department of Energy, the site contractor, sub-contractors, and the regulatory agencies to assure that these principles are adhered to. If they are not, poor management decisions are likely to be made, a great deal of money can be wasted, and the environment could be unnecessarily damaged.

II. ASSESSMENT OF VEGETATION AND SOILS (by E. F. Redente and T. McLendon, Department of Range Science)

A. Introduction

Vegetation and soils should be considered a critical component of any environmental assessment of a contaminated site. Depending upon site conditions, soils will commonly function as a primary storage medium for a contaminant and plants may accumulate pollutants over time. This accumulation may negatively affect growth and survival of plants. Additionally, contaminants accumulated by the plant are then available for bioaccumulation along the food chain.

B. Review of General Concepts

B.1 Goals of Sampling Designs

The design of a proper vegetation and soil sampling program depends on the purpose for which the results are to be used. Therefore, without an explicit purpose for the program, the sampling design cannot be developed for efficient data collection or proper interpretation of results. A detailed statement of purpose should be the first step in any sampling program.

B.2 Quality Assurance and Quality Control

The main objective of any quality assurance/quality control plan is to determine the quality of the reported data and insure that it is adequate for the intended use. Soils and vegetation, by their very nature, are extremely variable. Superimposed on this variability are other sources of variation or error that can be introduced into the final result by the sampling and analytical effort. A quality assurance/quality control program should be used to develop a system for assuring the quality of the results by attempting to provide control of the various steps in sample collection and the analytical process and to

provide adequate replication for statistically determining and quantifying the sources of variation encountered.

C. Sampling Procedure for Vegetation

The vegetation should be sampled with two designs, both utilizing the same procedures but sampling different types of sites. The first type to be sampled should be the general vegetation of the Rocky Flats Facility for the purposes of 1) providing background data to which changes in vegetation at specific SWMUs can be compared, and 2) broadscale monitoring of possible unexpected vegetation changes from activities (past and future) at the Facility. The second type to be sampled should be the vegetation associated with specific SWMUs to determine changes associated with natural causes and Facility activities.

The sampling procedure should also consist of two parts: 1) initial, and 2) continual. The initial sampling method should consist of identifying sampling locations along with data analysis to verify initial sampling accuracy. This part of the sampling procedure should be conducted only once and the time needed to complete this task will depend on the variation encountered in the vegetation. It is critical to the success of the overall vegetation sampling project that this initial procedure be conducted properly, and the level of expertise required to do so is much greater than that for the continual sampling. The continual sampling should be the procedure whereby sampling locations are monitored each year.

C.1 Initial Sampling Procedure

C.1.1 General Vegetation Sampling

Vegetation is dynamic in nature, i.e., it changes over time. Even those plant communities that are considered to be stable change over time, although changes within these communities are generally of a minor degree. However, this

condition of near stability may change for many plant communities in all parts of the world in the near future as a result of global changes in atmospheric and climatic conditions. It is imperative therefore, that an adequate background vegetation sampling design be used to gather data at the Rocky Flats Facility that can be used to monitor the effects of such changes on the vegetation and separate this variation from that which might be occurring because of Facility activities. Without such a background sampling design, or with an improperly used design, vegetation changes resulting from Facility activities may appear to be greater than they actually are. The use of the background vegetation sampling design will also provide a means to detect possible unknown contamination sites or unexpected results from known contamination sites.

The basic response units of the background vegetation sampling design (BVSD) are the specific plant communities found on the Facility. These communities have been mapped and described by Clark et al. (1980). Three sampling locations of each plant community should be selected in each cardinal direction from the geographic center of the Rocky Flats Plant. Sampling locations should be selected such that the three locations of each triad divide the distance between the geographic center and the outer boundary of the Facility into four approximately equal parts.

Within each of these 12 sampling locations of each plant community, one permanent line transect should be established. The line transect should be 50-m long and should be centered on a line connecting the three communities to the Main Plant center and should run along an axis parallel to the longest axis of the community at that location. The line transect should be marked in 2-m intervals with steel reinforcing rods. The five vegetation parameters listed in

Section C.2 (composition, production, rare or endangered species, chemical content, and photosynthesis rate) should be measured once per year along each permanent line transect. Data should be statistically analyzed according to the design and methods presented in Section E. The location of these sampling units, the placement of the permanent line transects, and the development of the statistical procedures for data analysis should be part of the Initial Sampling Procedure. The annual collection of data from the transects and the entry of the data for routine statistical analysis should be part of the Continual Sampling Procedure.

C.1.2 Specific Vegetation Sampling

The purpose of the specific vegetation sampling design (SVSD) is to monitor changes in vegetation that may be taking place on and near disturbed or contaminated sites and compare these changes to those taking place on similar nearby sites that do not have a history of disturbance or contamination. This comparison, along with the information provided by the BVSD, should provide the information required to determine if Facility activities are responsible for changes other than those associated with natural variation.

C.1.2.1 Basic Response Units

The basic response units of the SVSD should be selected sampling sites. Each SWMU should include at least one selected sampling site consisting of an area in which plants are present (i.e., not covered over with pavement or not continuously disturbed by human activities) and equal in area to at least 10% of the entire SWMU. More than one selected sampling site should be needed for a SWMU if: 1) the potential native vegetation of the area of the SWMU (based on Clark et al. 1980) includes more than one plant community, and 2) the total area of the most abundant community within the SWMU is less than 50% of the SWMU area.

If required, these additional selected sampling sites (one per plant community) should be located within the SWMU if the total combined area supporting that type of community accounts for at least 10% of the area of the SWMU.

Each sampling site within a SWMU should be located within the largest contiguous area of the appropriate potential plant community. A permanent line transect should be established (as in C.1.1) within the plant community in which the selected sampling site is located. The length of this transect should be 50 m or the maximum diameter of the plant community, whichever is less.

Each sampling site within a SWMU should be paired with a reference sampling site outside the SWMU. The reference sampling site of each pair should be placed within a plant community which is the same as that inside the SWMU (or would have been had it not been disturbed) and which has not been contaminated. The reference sampling site should include an area at least as large as that included within the SWMU and be of sufficient size and shape to allow for a transect of the same length. It should also have the same slope and aspect as the SWMU with which it is to be compared. Each transect pair should be unique, i.e., no reference transect should be paired with more than one inside transect.

The five vegetation parameters listed in Section C.3 (composition, production, rare or endangered species, chemical content, and photosynthesis rate) should be measured twice per year within each selected sampling site. Data should be statistically analyzed according to the methods and design described in Section E. The location of the sampling sites, placement of the line transects, and development of the statistical procedures for data analysis should be part of the Initial Sampling Procedure. The annual collection of data from the transects and the entry of the data for routine statistical analysis should be part of the Continual Sampling Procedure.

C.1.2.2 Disturbance Response Units

Some of the SWMUs have been physically disturbed. Such disturbance causes vegetational alterations, including future changes when the visible effects of the disturbance are no longer readily apparent. These changes are not the result of contamination by hazardous substances and should be recognized, measured, and separated from those possible changes attributed to the presence of contaminants. Otherwise, vegetational changes from contamination will appear to be greater than in fact they are.

The only way to recognize, measure, and separate vegetational changes due to physical disturbance is to monitor such changes on equivalent sites that are not contaminated and compare these results to sites that may be contaminated. However, these changes are time-dependent (i.e., they are influenced by time since disturbance and the environmental conditions that were present when they were disturbed), some of which (e.g., precipitation) vary from year to year. Unless the uncontaminated sites were disturbed in the same manner and at the same time that the sites that may have been contaminated were disturbed, these comparisons are only approximations. However, some aspects of secondary succession patterns are consistent between the same types of disturbances in spite of annual variations. Variations in vegetation that are the result of these patterns can reasonably be removed from the overall variation due to disturbance, thereby reducing one potential source of error. This reduces the probability of allocating too much variation to possible contamination (Type I error, Section E).

The following procedure should be used to estimate variation caused by physical disturbance. Plant communities within the sampling sites of SWMUs that have had significant physical disturbance should be determined and representative

examples of each of these plant communities should be selected in areas within the Rocky Flats Facility as distant as possible from points of contamination. Five examples of each pertinent plant community should be selected. At each of the five sites, 500 m^2 blocks should be physically disturbed in a manner similar to that imposed on the SWMU. These 500 m^2 disturbed sites should be termed Disturbance Sampling Sites. If there were major differences in types of physical disturbance within SWMUs (e.g, mixing of the entire soil profile rather than simply scraping off the topsoil or the deposition of large amounts of soil on top of existing vegetation), additional 500 m^2 blocks should be disturbed to match each type of physical disturbance within each pertinent plant community.

One 50-m permanent line transect should be established at each Disturbance Sampling Site in a manner similar to that of the BVSD. The five vegetation parameters listed in Section C.2 (composition, production, rare or endangered species, chemical composition, and photosynthesis) should be measured two times per year. The data should be statistically analyzed by the methods and design discussed in Section E. The determination of the required number and location of the Disturbed Sampling Sites, the preparation of the sites, the placement of the permanent line transects, and the development of the statistical procedures for data analysis should be part of the Initial Sampling Procedure. The annual collection of data from the transects and the entry of the data for routine statistical analysis should be part of the Continual Sampling Procedure.

C.2 Continual Sampling Procedure

Once the BVSD and SVSD sampling locations have been determined, routine vegetation sampling should be conducted. The following vegetation parameters (composition, production, rare or endangered species, chemical content, and

photosynthetic rate) should be sampled at the appropriate dates. Resulting data should be analyzed by the methods and design discussed in Section E.

C.2.1 Composition

Vegetation composition refers to the species and abundances of plants that are present on a given site. Composition should be measured by two methods: relative biomass and relative canopy cover. The method of estimating biomass will be discussed in the next section. Canopy cover data should be collected by use of the permanent line transects discussed in the previous section and from quadrats.

These data, meter by meter for each permanent line transect, should be recorded. The total number of centimeters of coverage by each species for each transect should be computed. Two additional variables, frequency of occurrence and number of species, should also be computed from these data.

C.2.2 Production

Production data should be collected at each sampling site by use of 0.5-m^2 (100 cm x 50 cm) quadrats. At each sample date, 10 quadrats should be randomly located along the permanent transects such that five quadrats are placed to each side of the transect. Before the quadrats are clipped, a species list should be made of the plants occurring within the quadrat and percent canopy cover will be estimated for each species. These data should provide the information required to adequately correlate the production data, taken from quadrats, and the composition data, taken from line transects.

For herbaceous plants, all aboveground plant biomass should be clipped by species. Litter should be collected and treated as a single species. For woody species, production should be estimated from height, cover, and stem circumference data. Total circumference of stems at ground level should be

measured (nearest mm). One branch from each woody plant found within each quadrat should be clipped, its length measured (nearest cm) and the circumference of the bottom portion of its stem measured (nearest mm). This clipped material should then be analyzed in the same manner as the herbaceous samples.

Regression equations should be developed for each woody species to predict biomass from length of stem and basal stem circumference. Data used to develop these equations should be the values collected for each species over all transects. New equations should be computed for each sample date.

Each sample of clipped material should be divided in half, forming two subsamples of each component, prior to drying. One subsample should be ultrasonically washed and the other sample left unwashed and the subsamples kept separate throughout the analysis to help separate physiological uptake from surface contamination.

C.2.3 Rare or Endangered Species

No rare or endangered plant species are known to currently be present at the Rocky Flats Facility. The species lists compiled from composition and production measurements should be checked after each sampling for rare or endangered plant species. Also, the BVSD sites should be surveyed twice a year to check for the presence of any rare and endangered plant species. A species list should be compiled for each of the 12 sampling locations of each BVSD plant community by an observer systematically walking over the entire area included within each sampling location and recording the presence of every plant species.

If any rare or endangered plant species are found on the Rocky Flats Facility, a 100 m^2 (10 m x 10 m) fenced enclosure should be constructed around the population and a non-destructive sampling design utilizing both quadrats and

transects should be developed to monitor the vegetation dynamics of that immediate location.

C.2.4 Chemical and Contaminant Content

Clipped material collected from the production sampling along each permanent line transect should be used to measure the chemical content of the vegetation. All samples, composited by species and by transect, should be stored for analysis. Sample material of the three most abundant species at each transect should be analyzed for content of major nutrients (N, P, C) and the possible presence of major radionuclide (plutonium, americium, and uranium) and metal (aluminum, barium, beryllium, cadmium, cobalt, chromium, lead, selenium, silver, strontium, thallium, zinc, mercury, nickel, and lithium) contaminants. In the event that abnormal levels of nutrients or contaminants are found, all samples of that species (all transects) and samples of all species from that transect collected during that year, the previous two years, and the following two years should be analyzed.

C.2.5 Photosynthetic Rate

Changes in vegetation composition and production may occur as a result of site contamination, however, these changes could develop relatively slowly. The more quickly such possible contamination is recognized, the more effective countermeasures can be, both to limit damage and to devise and implement corrective actions. Monitoring chemical content of the plants may accelerate the recognition process, since it is often the shifts in chemical content that cause physiological imbalances within the plants, which lead to shifts in vegetation composition and production. However, toxicity levels (and even normal variations) of many of these substances are not adequately known for most plant species. Hence, chemical content data may have limited value under some

conditions for early warning of danger from contamination. Under such conditions, shifts in plant physiological functions should provide the first warning of impending problems from contamination, and allow for more response time before the damage is reflected in production decline and composition changes in the vegetation. A physiological process common to all green plants is photosynthesis. Slight shifts in photosynthetic rate of a plant can alter health (growth and reproduction) and competitive ability.

The devices used to measure photosynthesis are usually referred to as gas-exchange systems. Photosynthesis is always a calculated parameter, determined from measurements of CO_2 concentrations and gas flows. Highly portable closed systems for measuring photosynthesis are available and ideally suited for conducting measurements in the field. One of the most popular self-contained closed systems is the LI-6200, Portable Photosynthesis System (LI-COR, Lincoln, Nebraska). It incorporates a flexible computerized data logger and calculates photosynthesis and leaf conductance along with measuring light, temperature, humidity and CO_2 concentrations. The system is designed for sampling large numbers of leaves under ambient conditions.

The three most abundant species within a SWMU, paired with the same three species in a control site outside the SWMU, should be sampled for the rate of photosynthesis. Four separate locations within each sampling site should be randomly located and the species to be sampled should be permanently marked for repeated measurements during the growing season.

Area of sampled leaves, sheaths, and stems should be determined using a portable leaf area meter. Regression analysis for leaf area and weights can be performed so that weight data can be converted to an area basis. Calculations and integrations should also be made for transpiration rates to determine water

loss from the two systems by transpiration. By knowing transpiration and net photosynthetic rates, it is possible to calculate water-use efficiency as affected by stress.

D. Sampling Procedure for Soils

D.1 Chemical and Contaminant Content

Soil samples should be collected from each SWMU and paired reference site. Sampling should be done separately in each soil type designated during soil mapping procedures. If there are two soil types within a SWMU, for example, then both should be sampled as a separate unit and paired with the same soil types in reference areas outside the SWMU.

Each SWMU and reference site should be sampled using a stratified random sampling approach. A selected number of sample locations should always be associated with vegetation sample locations so that correlations can be made between the chemical content of plants and the chemical content of soils. Each sample location should be sampled at three depths: 1) 0-5 cm, 2) 5-30 cm, and 3) 30-60 cm. Sampling should be conducted annually during the growing season.

Soil samples should be analyzed for radionuclides (such as plutonium, americium, and uranium), metals (such as aluminum, beryllium, cadmium, cobalt, chromium, barium, lead, selenium, silver, strontium, thallium, zinc, mercury, nickel and lithium), volatile organics (such as trichloroethylene, trichloromethane, carbon tetrachloride, dichloromethane, benzene, xylene, and methylene chloride), petroleum hydrocarbons and pH.

The probability for cross contamination among soil samples is high unless appropriate procedures are followed. The procedures provided in Section E should be followed to reduce sources of error that could be introduced during sampling and analysis.

D.2 Microbial System

The soil samples collected for chemical content (section D.1) should be split and half the sample used for chemical analyses and the other half used to assess the microbial community. Soil microorganisms play a critical role in the cycling of nutrients and in the flow of energy through their role in the decomposition process. A general assessment of the microbial community should provide valuable information on how the belowground system is functioning.

Each soil sample should be assessed for microbial biomass using lipid analysis (Vestal and White 1989). Determining the viable biomass of a microbial community provides an estimate of the amount of active microorganisms present. An analysis of the phospholipid component of the microbial community is a straightforward and sensitive measure of microbial biomass. In addition, the microbial community should be analyzed for metabolic stress. Many bacterial and eukaryotic cells store intracellular molecules during periods of stress. An analysis of these storage compounds can be used as indicators of the metabolic health of the community. The procedure should include an analysis for polybetahydroxy- alkanoates (PHAs) using gas chromatography after hot chloroform extraction, separation and methylation (Vestal and White 1989).

D.3 Soil Erosion

Monitoring of soil loss from wind and water erosion can be used as a measure of site stability. Long-term soil loss could lead to the exposure of contaminants that are presently buried.

Permanent transects should be randomly located on SWMUs with slopes of 20% or greater to monitor soil loss. The most efficient and accurate method for estimating soil erosion is a technique described by Toy (1983). Measurements along permanent transects should be taken annually.

E. Sampling Design

E.1 Quality Assurance/Quality Control

E.1.1 QA/QC Procedures

An adequate quality assurance/quality control (QA/QC) program requires the identification and quantification of all sources of error associated with each step of an environmental evaluation program so that the quality of resulting data will be known. The components of error include those associated with sampling, sample preparation, extraction, analysis and residual error. For monitoring relatively heterogeneous components of an ecosystem such as soils and vegetation, the sampling component of variance will usually significantly exceed the analysis component.

Another important aspect of QA/QC is auditing. The purpose of an audit is to insure that all aspects of the QA/QC system planned for the project are in place and properly functioning. This includes all aspects of field and laboratory efforts. Whenever a problem is identified, corrective action should be initiated and pursued until corrected. Chain-of-custody procedures and raw data should be checked and results from blind samples, routinely inserted for laboratory analysis, should be reviewed.

An audit of the overall QA/QC plan for sample documentation, collection, preparation, storage and transfer procedures should be performed before sampling begins. This step is necessary to critically review the entire sampling program to determine the need for any corrective action prior to initiation of the work.

The project leader of each sampling phase should be responsible for assuring that all members of his team have adequate training and experience to carry out their assigned tasks in a satisfactory fashion. This is normally

accomplished through a combination of required classroom training, briefings on the specifics of the project and field training exercises.

The most highly developed aspect of quality assurance in support of environmental monitoring programs has been for the analytical procedures. However, such an approach is not acceptable in cases where the material being sampled (e.g. soils, sediments and vegetation) is not homogeneous. Therefore, quality assurance of the analytical results is necessary but not sufficient for assessing total sample variability. The analytical errors may account for only a small portion of the total variance. In view of this, a more comprehensive quality assurance program is mandatory for the sampling effort.

In each case where environmental monitoring is determined to be necessary, administrative or legal actions are likely to be taken on the basis of an evaluation and interpretation of the resulting data. The consequences of taking or not taking action must be clearly understood before it is possible to establish an allowable confidence band for quality assurance of the data. After weighing and evaluating the consequences, a value judgment should be made by a responsible official concerning the acceptable probability of making a Type I or a Type II error. It is not possible to design a meaningful quality assurance program until this step has been taken.

Type I error is the case where a hypothesis is rejected when in fact it is true. Type II error is the case where a hypothesis is accepted when in fact it is false. The Type I error is most frequently encountered in statistical tests used in the literature. In environmental monitoring, however, the Type II error is more important. The Type II error could lead a manager to conclude that a cleanup of some area is not necessary when in fact the action levels are being exceeded and cleanup is necessary.

E.1.2 Location of Sampling Sites

The location of the sampling sites will depend on whether the sampling is random, systematic, or some combination of the two. In the event that it is impossible to obtain a sample at a randomly selected location, a sample should be obtained from the closest available alternate site. For example, the selected site may be below an asphalt cover. In a case such as this, any errors introduced by moving to the closest alternate site are not likely to unduly influence the overall results.

The movement of contaminants over the surface of a site and through the soil mass may be strongly influenced by topographic features, soil type, geologic formations, and vegetation. If any of these factors are important, the sampling design should be stratified in order to include each important factor. For example, a liquid pollutant deposited on a hilltop will move downslope. Maximum concentrations are likely to occur in low areas as opposed to ridgetops. Stratification of such an area into three strata (ridgetop, hillside, and valley floor) is recommended. This design allows the analysis of variance to remove the variation due to these three strata from the total error term, thus reducing the estimated sample variance.

E.1.3 Sample Collection

An important aspect of the QA/QC plan deals with sample collection. This aspect of the monitoring program should be designed to meet the specific objectives of the study. Improperly collected samples can void the entire study.

Estimates should be made of the components of the variance or error associated with each element of the sample collection methods and procedures used from the data generated by the study. It is recommended that a minimum adequate approach be sought consistent with the objectives of the study, the resources

available and the designated required levels of precision and confidence. Also, an effort should be made to establish some criteria for estimating, after the fact, whether or not the sample collection elements of the QA/QC plan and objectives were satisfactorily achieved.

A minimum sample volume should be specified for samples requiring laboratory analysis. Sample size will be dictated by the method and required sensitivity of the analysis. The method of collecting samples should take into consideration the required depth of sampling as well as required amounts when sampling materials having a depth component like soils or sediments.

The sample collection device should be adequate to obtain samples to the required depth. The sampling device should be carefully cleaned between each use to avoid cross contamination of samples. Suggested cleaning methods are given by EPA (1982).

Frequently when collecting environmental data, anomalies will be encountered. These anomalies should be documented and noted in field manuals to assist in data interpretation.

E.1.4 Sample Handling

Aspects of the QA/QC plan dealing with sample handling, including the transfer of the sample from the collecting device to a suitable container, transportation of the sample, and the preparation of the sample for analysis are as important as collection of the sample. The QA/QC plan should address the following: 1) type of container material, 2) cleaning procedures for the containers, 3) decontamination procedures for sampling instruments and equipment used in sample preparation, 4) labeling scheme and log book entries, 5) chain of custody procedures, 6) sample preparation procedures in the field and laboratory (EPA 1979).

Spiked samples and blanks should be an integral part of the analytical process to measure the internal consistency of the samples and to provide an estimate of the components of variance and the bias in the analytical process.

Spiked samples are prepared by adding a known amount of reference chemical to one of a pair of split samples. The results of the analysis of a spiked sample compared with the non-spiked member of the split measures the recovery of the analytical process and also provides a measure of any analytical bias. Spiked samples are difficult to prepare for materials like soil and vegetation because of the problem of mixing. The addition of the spike solution to an extract in the laboratory avoids the mixing problem.

Blanks provide a measure of various cross-contamination sources, background levels in the reagents, decontamination efficiency and any other potential error that can be introduced from sources other than the sample. For example, a trip blank measures any contamination that may be introduced into the sample during shipment of containers. A field blank measures input from contaminated dust or air into the sample during field collection. A decontamination blank measures any chemical that may have been in the sample container or on the tools after decontamination is complete.

QA/QC programs for analytical laboratories are widely used and accepted. These programs are strongly oriented toward the analytical process, not toward the sampling effort. It is suggested that 15 to 20% of the total analytical work load be dedicated to the quality control program. Of this, 25% should be allocated to the laboratory effort and 75% should be allocated to the sampling effort.

E.2 Statistical Procedures

E.2.1 Sample Size

The vegetation and soils of the Rocky Flats Facility should be sampled, i.e., only a portion, not the entire population, of the vegetation and soils will be measured. Therefore, conclusions drawn from sampling are based on the assumption that the samples adequately represent the entire population. If this assumption is false, then the conclusions may also be false and decisions made based on these conclusions could be inadequate or incorrect. Two critical requirements that should be constantly adhered to are that 1) the sampling design be correct, in design and implementation, and 2) that the sampling be adequate, in number and type. If these two requirements are met, the conclusions reached from the data should be valid (within the confidence levels of 95% or 99%) for the population as a whole and thus useful as a management tool.

Sample size, for populations that have Normal statistical distributions, is dependent on only two parameters at any given probability level: 1) the accuracy desired for the estimates, and 2) the variation within the population. The greater the degree of accuracy desired, and the more variable the population, the more samples are required. If the values of these two parameters (or reasonable estimates of them) are known, the number of samples needed to achieve that degree of accuracy, at the probability level designated, can be determined by the formula:

$$n = 3.84(var)/L^2$$

where

n = number of samples

var = variation of the population

L = accuracy (population mean $\pm$ L)

for the 95% probability level (i.e, 95% of the time, this number of samples will result in this accurate of an estimate). For the 99% probability level,

$$n = 6.66(var)/L^2$$

(Snedecor and Cochran 1967). However, the variation in the population is seldom known, since all individuals of the population would have to be measured to determine it. Therefore an estimate, s^2 (variation in the sample), is used instead. When s^2 is used instead of the variance, the constants 3.84 and 6.66 must be modified also. The number used is the t-value (Student's t-value, taken from a table of t-values at n-1 degrees of freedom) squared. Using, for example, the number of examples of a given plant community in the BVSD, n = 12 and the corresponding t-values are 2.20 at the 95% level and 3.11 at the 99% level. The constants in the formulas then become 4.84 (instead of 3.84) and 9.67 (instead of 6.66).

It is not possible to determine the number of samples required to achieve a given degree of accuracy before the population is sampled, since the variance of the sample (or the population) is unknown. Once the first sampling takes place at a given intensity (number of samples), then an estimate of the accuracy of the sampling can be made. Twelve samples per plant community have been suggested for the initial BVSD design. This may or may not be sufficient to achieve a desired degree of accuracy. If it is not sufficient, and this can be determined only after the first sampling period, then additional sample sites will need to be added (or accuracy of the estimates decreased). On the other hand, that number of samples may be more than sufficient to achieve the desired degree of accuracy. In that case, the number of samples could be reduced or the degree of accuracy increased. A similar situation exists for the SVSD and soil sampling designs. As data accumulate, statements as to sample adequacy and

accuracy of estimates can be made. The determination of adequacy of number of sample sites will be made by the Initial Sampling Procedure (Section C.1).

E.2.2 Paired t-Tests

Paired t-tests should be conducted on all data sets as one method of detecting differences due to annual variation, physical disturbance, and possible contamination. The statistical distributions of each variable (separately by species) should be compared to the Normal Distribution by use of an appropriate test, such as the Chi-square Goodness of Fit Test. Appropriate statistical transformations should be made for those variables whose distributions differ significantly (95% level) from that expected from a Normal Distribution, and the transformed distribution checked against the Normal Distribution.

For the BVSD data, the values of each variable, by species, for each year should be tested against the values for that variable from the previous year. This will give an indication of significant changes occurring from year to year. If no significance is indicated for that variable, that year's data should be compared to the data from two years previous. This procedure should be continued until either significance is indicated or the set of possible comparisons is exhausted. The lack of any significant differences indicates stability for that variable for that species over the time period sampled. This would suggest that there is lack of any background changes taking place during the period sampled and that any significance found in the SVSD can be attributed to disturbance (physical or contamination). However, if significant differences are found in the paired t-test analysis of the BVSD data, there exists the probability (95% level) that there are background changes taking place.

Year-by-year paired t-test comparisons can not be made for the SVSD data set because of lack of multiple observations (there is only 1 transect per plant

community per SWMU). If necessary, procedures for making approximate comparisons can be developed.

E.2.3 Multivariate Analysis

The paired t-test analysis will allow very specific statements to be made regarding vegetation dynamics and the possible effects of contamination on the vegetation. Statements will be possible as to the response dynamics of individual plant species and chemical components of the species, in addition to general compositional changes. However, there is a weakness to univariate analyses such as t-tests. Statements made about the vegetation as a unit are composite statements, and therefore statistically artificial, since the analyses were conducted on individual species. Bias and information loss generally result from such composite statements. Multivariate analyses do not suffer from this weakness. In these analyses, each species variable is included in the same analysis. The response unit becomes the sum of these individual variables (species), therefore the vegetation itself is being tested. The major limitation of multivariate analyses is that since the species variables are not being tested, less can be said about them. However, this is the strength of univariate analyses. Therefore, the combination of the two types of statistical analysis gives a more complete understanding of the dynamics of a vegetation data set.

The multivariate statistical technique to be used should be stepwise discriminant analysis (Lachenbruch 1975, Matthews 1979, McLendon and Dahl 1983). The first use of discriminant analysis should be to test differences in plant communities in the BVSD. The vegetation classification system of Clark et al. (1980) is a traditional system based on subjective classification of observed differences in distribution patterns of plant species. Their units, the plant communities, should be used as the basic response units in the BVSD, which is a

sampling design that must yield quantitative data to be used in rigorous statistical analysis. The initial assumption is that the qualitative classification units of the system of Clark et al. (1980) can be used as a basis for a quantitative system (BVSD). This assumption should be tested in order to determine how different the units are quantitatively. Depending on these results, fewer or more units (plant communities) might be required in order to yield the desired degree of accuracy. The plant communities can be tested using the data collected from the BVSD during the first year. A separate discriminant analysis should be run for relative canopy cover, relative biomass, and each contaminant of concern. These could be run as a single analysis, but separate analyses should give a more complete view of possible differences. Since discriminant analysis assumes the variables have a Multivariate-Normal distribution, only those variables whose distributions were not found to differ significantly from Normal should be used in the analysis. The discriminant analysis should test the significance of differences between groups (plant communities) based on the mean values of the plant species variables. If certain groups are not significantly different from each other, they can be combined and the number of subsequent BVSD response units (and therefore sampling sites) can be reduced. It is also possible to determine if the groups should be further separated. If so, additional BVSD response units should be selected. This set of discriminant analyses should be included in the Initial Sampling Procedure.

Once these initial analyses are completed and the number of BVSD response units determined, discriminant analysis can be used each year to test differences in BVSD response units to help monitor background vegetational changes. This set of discriminant analyses should test differences between groups (plant communities) and years based on variation in a single vegetation attribute (e.g.,

relative biomass, nitrogen content, plutonium content) within all species (those with distributions approximately normal). A separate discriminant analysis should be run for each attribute. In each analysis, all appropriate data per transect per year should constitute an observation. Grouping should be by plant community and year. If significant differences are occurring from year to year, the year groupings (or some sub-set of them) within a given plant community will differ significantly from each other. If only plant community groups differ from each other, there are no significant year-to-year changes in the vegetation dynamics. Responses of individual observations (transect locations) can also be tested. Significant differences within this component would indicate that changes are taking place within a given plant community in relation to location, which could be in relation to either cardinal direction (natural variation) or sources.

SVSD data sets should be tested by discriminant analysis in a similar manner. For those SWMU sites where there has not been significant physical disturbance, a discriminant analysis should be run testing differences between transects (inside and outside) and years based on all vegetation attributes for all appropriate species. Grouping should be by season, transect, and year. Significance due to year would indicate either natural changes or changes due to contamination that take time to be manifested. Significance due to transect would indicate contamination if the paired transects were significantly different and natural ecological differences (plant community) otherwise.

Those SWMU sites where there has been significant physical disturbance should be analyzed by discriminant analysis in a similar manner except each transect should be paired with the appropriate disturbance site transect and year would be based on year since disturbance, not year of data collection.

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III. ASSESSMENT OF TERRESTRIAL AND RIPARIAN WILDLIFE (by A. W. Alldredge, Department of Fishery and Wildlife Biology)

A. Introduction

A major factor influencing the presence of wildlife in a given area is the availability of suitable habitat. The number of individuals per unit area of available habitat is termed ecological density. Although ecological density is often a parameter measured in impact analysis, determining it is a difficult process because of spatial and temporal variability associated with habitats and wildlife populations. Furthermore, there are few techniques available to accurately determine the density of small mammal and bird populations. A simple density figure, however, may not reflect actual impacts and such a figure should be evaluated with individual survival data as well. The presence of wildlife species may also be influenced by anthropogenic contaminants that influence survival and reproduction of organisms. An environmental assessment for wildlife populations must consider all these factors. Because of the great variability associated with wildlife populations and their habitats few sampling procedures possess much statistical rigor. Included in this report are methods that range from general qualitative inventory, to the best techniques available for acquisition of quantitative data. It is advisable to at least inventory species at SWMUs and, where feasible, to establish a sampling protocol that will provide statistically valid documentation of population changes in a spatial and temporal context. Although there has been a recent trend in selecting single "indicator species" to monitor as representatives of land use impacts on whole systems, there are no data to suggest that a single species is inextricably linked to the entire system, or that the response of a single species depicts the response of

the entire system. Therefore, major segments of entire communities should be monitored.

Wildlife considered in this section are birds and mammals. The publication, "Ecological Assessment of Hazardous Waste Sites: A field and Laboratory Reference" (EPA 1989a) outlines adequate procedures for sampling terrestrial invertebrates and ectotherms. Guidance for development of this section of the report was provided by that document as well as Risk Assessment Guidance for Superfund Volume II Environmental Evaluation Manual (EPA 1989b).

B. General Considerations

Because wildlife populations are intimately linked to habitat, wildlife studies should be correlated with vegetation studies. In general, if suitable habitats are available, or if disturbed sites can be successfully restored to provide habitat, wildlife populations will establish themselves in these habitats. Therefore, wildlife sampling areas should be conjoined to those areas selected for vegetation sampling. This approach emphasizes the wildlife-habitat linkage and, because detailed habitat information will be available from vegetation sampling, the interpretation of wildlife data will be enhanced. However, the assignment of causal factors to differences observed in populations of plants and animals cannot be made strictly from field sampling data. Evaluation of published scientific literature, toxicity tests and consultation with technical experts will be needed prior to drawing conclusions regarding causal mechanisms.

Because of the need to keep impacts on wildlife populations from data gathering to a minimum, the suggested approaches involve non-destructive sampling. Exceptions are cases where tissue samples are necessary to assess trophic transport of contaminants and potential bioaccumulation.

Although habitat at the Rocky Flats Facility is the property of the US Department of Energy, wildlife is considered the property of the State of Colorado and is under jurisdiction of the Colorado Division of Wildlife (CDOW). Prior to any trapping or collecting of wildlife at Rocky Flats, an appropriate collector's permit must be obtained from CDOW.

Because of the relative homogeneity of Rocky Flats environments and the variability inherent in wildlife populations, it may not be necessary to sample each SWMU intensively. As a first approximation for ascertaining which sites must be sampled, it is suggested that the site history, including types and levels of contaminants and surface disturbance history, and vegetation community be used as descriptors. Secondly, the size of the SWMU should be considered. SWMU's smaller than 0.25 ha are too small to be adequately sampled for wildlife populations. For SWMU's larger than 0.25 ha, representative areas may be sampled to the exclusion of others if site history and vegetation communities are similar. It is emphasized, however, that comparable sites should be sampled as replicates to increase validity of statistical tests.

C. Quality Assurance and Quality Control

The quality of conclusions drawn from environmental data is largely affected by the quality of data collected for these assessments. Contractors conducting wildlife studies must be familiar with mammalian and avian field identification, possess the appropriate equipment (live traps, etc.) for population sampling and be well versed in the application of recommended analytical and statistical procedures. Recommended references for mammal identification are Lechleitner (1969) and Armstrong (1975) and for birds the National Geographic Society (1983) and Peterson (1961). Methods for capture-recapture sampling methods are discussed in White et al. (1982) and Burnham et

al. (1987). Sampling methods for avian populations are discussed in Hutto (ND) and Ralph and Scott (1981). Multivariate statistics have been useful in wildlife habitat studies, but consultation Rexstad et al. (1988, 1990) prior to application of these techniques is recommended. Accurately ascertaining impacts of Rocky Flats activities on resident wildlife is a process that must be conducted over time. Quality results cannot be obtained from poorly designed, short term studies.

D. Large Mammal Populations

Large mammals utilizing environs of the Rocky Flats Facility include mule deer (Odocoileus hemionus), white-tailed deer (O. virginianus), coyotes (Canis latrans), foxes (Vulpes vulpes and possibly Urocyon cinereoargenteus), and badgers (Taxidea taxus). Elk (Cervus elaphus) have been observed at Rocky Flats but are only occasional visitors. Because these animals range over large areas relative to the size of the Rocky Flats Plant and the SWMU's, it would be difficult, if not impossible, to design studies to evaluate the impact of each SWMU on populations of these mammals. With the exception of deer, it is recommended that inferences regarding impacts to the remaining large mammals be drawn from data for small mammal and bird populations. For example, using documented levels of contaminants in small animals at Rocky Flats and published literature, upper bounds for the body burdens of larger predators could be estimated. Deer are a conspicuous part of the Rocky Flats animal community and are possibly one of the best integrators of environmental perturbations across the facility. Because of these factors, deer populations should be given consideration in the environmental evaluation procedure.

An intensive investigation on deer ecology at Rocky Flats is currently underway. The purpose of this investigation is to collect data that can be used

in assessing the impacts of Plant operations on deer and in predicting impacts on deer from remedial actions and alternative uses of the Plant environs. Specific objectives of this work are to document seasonal habitat use patterns of deer at the Plant. These data will indicate the relative use of SWMU's, as well as the importance of specific habitats and the Plant site as a whole. Using these data, existing published information, and measured contaminant data, body burdens for selected contaminants in deer can be evaluated. This study will also document dispersal of deer from Rocky Flats in order to assess the potential that deer may either spread contaminants to surrounding environments, or represent a pathway for transmission of contaminants to humans. Additional objectives of this investigation are to elucidate population dynamics of the Rocky Flats deer herd.

E. Small Mammal Populations

Small mammals are predominantly herbivores and most of those present at Rocky Flats are ground dwelling species. The close association of these small mammals with contaminated soils and vegetation, their important link in food webs, and their limited mobility relative to large mammals and birds, underscores their importance in environmental evaluations. For assessment purposes at Rocky Flats, there is little merit in assessing lagomorph or chiropteran (rabbit or bat) populations. Therefore, the focus of this section of the manual is on the small ground dwelling mammals such as mice, voles and ground squirrels.

Three basic considerations are included in the suggested approach to evaluation of small mammal populations: 1) size of the SWMU, 2) contaminants present, and 3) perceived impacts.

The purpose for which the collected data are to be used influences the selection of sampling regimes for evaluating small mammal populations. To assess

the status of small mammal populations and impacts of SWMU's on small mammals, each SWMU selected for analysis should be paired with a comparable reference area. It is recommended that these reference areas be the same areas selected for the vegetation community analysis (Section C.1.2.1. of this report). Care must be exercised to insure that investigators collecting data on small mammal populations do not impact vegetation study plots.

Assessment of impacts on small mammal populations requires that both population density and individual survival be estimated. Estimating only one of these parameters could be misleading and result in erroneous conclusions. Assessment of both density and survival rates requires intensive field sampling and detailed statistical analysis. Prior to deciding what sampling protocols should be used, contaminant levels, SWMU size, and management decision criteria need to be evaluated. Detailed methodology for density and survival estimation are presented in White et al. (1982) and Burnham et al. (1987). Technical expertise should be sought in the design and execution of these types of studies.

F. Contaminants

In areas where biologically active materials exist, small mammals should be collected and contaminant body burdens measured. Without some knowledge of the variance associated with body burdens, the calculations of statistically valid sample sizes are not possible. Collection of preliminary data would allow a variance estimation, and sample size could then be calculated for a pre-determined level of confidence. A detailed discussion of sample size and confidence is presented in "Ecological Assessment of Hazardous Waste Sites: A Field and Laboratory Reference," (EPA 1989a).

Evaluation of contaminant body burdens necessitates comparison of data obtained from animal tissue specimens on contaminated areas to those obtained

from reference sites. It is recommended that animals be obtained from areas that have been selected as vegetation reference sites. These sites, however, should not be the same areas where long term population analysis investigations are being conducted.

When collecting samples for contaminant body burden analysis, care must be taken to insure that there is no cross contamination and that animal samples are not contaminated with soil or vegetation material. Live traps should be used to collect samples for contaminant analysis. Captured animals should be transferred to a clean "kill jar" and euthanized with metafan. Once euthanized, animals should be individually bagged and marked with the following data: date, species, sample site and collector. Residue from soil, feces and vegetation residue should be cleaned from the traps daily.

Analysis of collected samples will vary depending upon the contaminant. Generally, bone, liver, kidney, brain, and muscle are the tissues that should be considered for analysis. During dissection, care must be taken to eliminate cross contamination. Fur of ground dwelling small mammals may contain contaminants from soil that are not actually in body tissues. Fur and gut contents, in addition to internal tissues, should be analyzed. In general, when predators consume small mammals, the entire carcass is eaten. Thus, it is necessary to know entire body burdens as well as selected tissue burdens in prey animals when assessing potential contaminant transmission to predators. To insure quality control, blind duplicates of selected tissue samples should be submitted to laboratories for contaminant analysis. It is also recommended that a reference collection of samples be maintained at least until analytical results have been received and evaluated.

G. Consideration of the Size of Waste Management Units

The best method for obtaining data on small mammal population density is a large trapping grid and the use of capture\recapture methodologies. A minimum grid size of 4.5 hectares is recommended (this assumes a grid consisting of 15 trap stations on a side, with 15 meters between each trap station) to obtain reliable data on small mammal population density. Estimation of survival rates can be made from these same trapping grids, but consideration must be given to the sample size necessary to detect ecologically important differences.

Regardless of SWMU size, live traps should be used to collect all population data. Sherman live traps are recommended for all small mammal trapping. Additional species such as shrews might be captured using snap and pit-type traps, but these destructive methods should be avoided if possible. There should be two traps at each station, and traps should be baited with a mixture of oatmeal and peanut butter. Traps should be set in the late afternoon and checked and sprung in the early morning. This approach will miss some diurnal small mammals, but most small mammals are nocturnal or crepuscular. If traps remain open throughout the day, they must be frequently checked or captured animals will die from heat stress. Trapping should be conducted for a minimum of six consecutive days and paired SWMU's and reference areas must be trapped over the exact same time interval.

Data collected from each trapped mammal must include species, age (juvenile or adult), reproductive status, and identification number. Identification of individual small mammals is essential to estimate population densities and survival. Various techniques are available to mark individuals. Toe clipping is often used, but the success of this technique is dependent upon the diligence of the investigator. For burrowing small mammals, toe clipping may impair survival. Small mammals may be individually tagged by placing fish fingerling

tags in the ears. If this approach is selected, tags should be placed in both ears since tag loss is often a problem. Passive Integrated Transponder (PIT) tags are the most reliable method for individually marking small mammals in long term studies. These tags are inserted sub-cutaneously in small animals and thus cannot be lost. With this method, there is little probability of mis-identification, and the tag does not appear to influence survival.

G.1. SWMU's Smaller than 4.5 Hectares

Some SWMU's at the Rocky Flats Facility are so small that sampling for small animals is precluded. If the SWMU is suspected to contain biologically active contaminants, trapping is advised in that area and the area immediately surrounding it to obtain small mammal tissues for contaminant analysis. For SWMU's smaller than 4.5 ha, but larger than 0.25 ha, a qualitative assessment of small mammal populations is recommended. Sampling intensity will vary depending on the size of the site, but placing live traps at 10 meter intervals on these smaller sites is recommended. At each trap station, two live traps should be placed; traps should be baited and checked as recommended above. Because of the influence of the edge effect on small trap grids, data obtained from this approach should be used only in a qualitative sense and compared as such to reference areas. Trapping many of these smaller sites may need to be done only once, for example in late summer. Depending upon capture success, it might be possible to obtain survival estimates for small mammals on some of the smaller SWMU's, but interpretation of survival data without population density estimates may be difficult.

G.2. SWMU's 4.5 Hectares and Larger

When the area of the SWMU is 4.5 ha or larger, a trap grid consisting of 15 trap stations on each side should be established (total 225 trap stations).

Trap stations should be 15 meters apart with two traps set at each station. Traps should be baited and checked as recommended above. Larger sites afford greater potential to accurately assess impacts. Because of this, permanent trapping grids should be established in these areas and in paired control areas, and these grids should be trapped over a number of years. Where possible, replicate trapping grids should be established. As recommended above, paired control areas should be the same areas selected for vegetation sampling. An annual trapping in late summer should be adequate to estimate population density. It is essential, however, that each set of paired plots (SWMU and reference site) be trapped during the same time interval. Analysis of data from these trapping grids should be conducted according to White et al. (1982). Differences in population density and structure can be ascertained using this approach. Survival estimates can be obtained for small mammals on these sites from monthly trapping sessions over a period of six months. Trapping would need to be conducted on both the SWMU and reference site simultaneously, but this intense effort would probably need to be conducted only once depending upon variability of the data.

If differences in population density and survival between SWMU's and references areas are detected, the assignment of causal relationships may be a complicated task involving additional study and consultation. In cases where significant differences are detected, data should be evaluated in the context of associated habitat data obtained from vegetation studies and contaminant data. If toxic and/or biologically active contaminants are present, small mammals should be sampled for contaminant levels. Such sampling should not be done on the permanent sampling grids. Contaminant levels alone may be of little value

in assigning causal relationships, and additional laboratory studies may be required to evaluate toxicity and effects on reproduction.

Surface disturbance and successional dynamics of the vegetation community may be responsible for observed population differences. Data for small mammal populations in reference areas with similar site disturbance history would facilitate interpretation of population trends. If differences in small mammal populations are detected, replicate disturbance areas should be established and sampled. Such an area would yield data relative to successional dynamics in both the autotrophic and heterotrophic communities at Rocky Flats.

H. Avian Populations

In his review of landbird census methods, Hutto (ND) concludes that, "there is no 'best' method independent of a person's study goals," and further he adds, "...the one generalization that seems fair to make is that most popular methods for calculating bird density should be abandoned." Based upon these conclusions, it is not recommended that attempts be made to estimate bird densities at Rocky Flats. Simple relative counts using the same methodologies on SWMU's and reference sites will provide adequate data relevant to avian populations. Data obtained from counts will range from mere presence/absence information to relative abundance. Capabilities of the observers collecting avian population data often represent a significant bias. It is usually essential that the same observer conduct surveys on SWMU's and paired reference areas.

For large avian species, such as raptors, general surveys of the entire Rocky Flats Plant should be conducted seasonally. These surveys must be conducted seasonally to tabulate information on yearlong residents, breeding birds, winter residents and migrants.

Smaller birds can be surveyed using one of several techniques. Recognized

authorities use a variety of approaches, each having its own set of assumptions and limitations. Medin and Booth (1989) and Scott et al. (1982), for example, used the Williams spot-map method which has been recommended by the International Bird Census Committee (1970). Sarzo and Balda (1982) discussed territory mapping, the line transect method, and the variable circular plot, and concluded that, "in areas of larger, more uniform, and less complex habitats the line transect or strip count method might be considered an acceptable alternative because it allows the coverage of larger areas per unit of time." Because of the comparatively homogeneous environs of Rocky Flats, adequate data on relative abundance of avian species can be obtained from line transects, strip counts, or variable circular plots (Reynolds et al. 1980). These techniques do not provide good estimates of density, but they do provide a relative index of bird numbers. Such an index should be adequate for environmental assessments at Rocky Flats.

The same technique must be employed on both SWMU's and a comparable reference site. The reference site should be the same paired reference site as that selected for the vegetation studies. Surveys for smaller birds should be conducted during May and June to ascertain populations of breeding birds and during winter for detection of winter residents.

I. Riparian and Lacustrine Environments

Riparian and lacustrine environments are among the most diverse, least abundant and most valuable for wildlife. They are often the environments most frequently impacted by human disturbance. Contaminant issues are no exception. At Rocky Flats, decisions regarding surface water management can create or eliminate wetland environments. The Walnut and Woman Creek drainages at Rocky Flats contain wetland habitats that are potentially impacted. Rock Creek is

potentially a useful reference site as is a small pond in the southeast segment of the Buffer Zone.

Wildlife populations in wetland habitats should be monitored using the same techniques as outlined above. Emphasis should be placed upon breeding birds during May and June. Wildlife population data should be evaluated in conjunction with contaminant data in water, substrate and water-based forage items in order to evaluate potential transfer to wildlife.

Waterfowl represent a potential contaminant transport pathway to humans. Because they are easily observed, and wetlands are limited at Rocky Flats, waterfowl abundance can be estimated from simple surveys. These data should be obtained seasonally to account for residents and migrants. Equilibrium body burdens of some contaminants in waterfowl can be estimated roughly using data for contaminants in water, sediments, and forage. If estimated contaminant levels in waterfowl are high, we recommend collecting birds to ascertain actual levels. Samples should be taken during August and September to collect birds that have spent the majority of summer at Rocky Flats. Another approach is the use of introduced, wing-clipped waterfowl to assess the uptake and toxicity of contaminants.

J. Threatened and Endangered Species

There are no records of threatened or endangered species at Rocky Flats. Bald eagles (Haliaeetus leucocephalus) have been observed flying over Rocky Flats, but there are no nesting populations or winter concentrations in the immediate area. It is recommended that field personnel continually watch for rare and endangered species but no special sampling procedures for this class of animals is proposed. In the event that rare and endangered species are

encountered, both the U.S. Fish and Wildlife Service and the Colorado Division of Wildlife must be informed.

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IV. ASSESSMENT OF THE EFFECTS OF CONTAMINANTS ON AQUATIC ECOSYSTEMS

(by W. H. Clements, Department of Fishery and Wildlife Biology)

A. Objectives

The purpose of this section is to recommend site-specific procedures for evaluating the ecological impacts of chemical and radioactive contaminants on aquatic systems at Rocky Flats. Emphasis will be placed on assessing structural and functional alterations in these systems, measurement of contaminants in biotic and abiotic components, and estimating the acute and chronic toxicity of these contaminants. The recommended approach will require a highly coordinated field and laboratory assessment of: 1) what contaminants are present; 2) the concentrations of these contaminants in water, sediments, and organisms; 3) effects of these contaminants on natural populations and communities in the field; and 4) lethal and sublethal effects of these contaminants on organisms in the laboratory.

B. Background

The proper design of any ecological field investigation requires decisions regarding which variables should be measured, appropriate sampling procedures, the sampling design, and appropriate descriptive and inferential statistics. Green (1979) provides an excellent review of sampling design and statistical methods specifically for environmental biologists involved in assessing the impact of contaminants. The choice of variables, sampling procedures, and determination of necessary sample size is discussed below.

B.1. Structural and Functional Variables

Assessment of the ecological impact of contaminants on aquatic systems involves measuring changes in either structural or functional characteristics. The relationship between these two classes of measurements is discussed by Cairns

and Pratt (1986). In general, structural measurements involve counts of organisms (abundance, number of taxa, etc.) whereas functional measurements involve rate processes (primary productivity, detritus processing, nutrient cycling). The decision regarding which class of measurements is most appropriate for assessing the impacts of contaminants is controversial. There is some evidence suggesting that measurement of ecosystem function is more ecologically relevant; however, owing to the functional redundancy of ecosystems, greater variability of functional parameters, and the comparative difficulty in measuring these parameters, the usefulness of functional variables for detecting effects of contaminants may be limited. Although Schindler (1987) concluded that variables reflecting function in aquatic ecosystems (primary production, nutrient cycling, respiration) were relatively poor indicators of early stress and that structural measurements were more useful, it is recommended that both structural and functional parameters be included in assessment of the effects of contaminants at Rocky Flats.

B.2. Determination of Necessary Sample Size

The number of samples required to demonstrate significant differences between reference and impacted sites is of critical importance in the design of field assessments. Because biological parameters show considerable spatial and temporal variability, a large number of samples is often necessary. The number of samples required to detect differences among locations is a function of sampling variability and the relative degree of precision required by the investigators. Several algorithms are available to estimate sample size based on these variables. Green (1979) presents the equation:

$$n = [s.d.\ (t)/d\ (x)]^2$$

where

n = required number of samples

s.d. = standard deviation

t = Student's t-statistic

d = required precision

x = estimated mean of the population.

The mean and standard deviation in the above equation are estimated by preliminary sampling. The required degree of precision (d) is a subjective value based on the investigator's knowledge of what constitutes an ecologically significant difference between two populations. An investigator must decide, for example, if a 20% difference in the number of species between reference and impacted sites is biologically and ecologically relevant.

Logistical considerations, such as cost of sampling, length of time to collect and process samples, etc. will limit the number of samples collected. Because the balance between collecting the appropriate number of samples, sampling precision, and sampling cost is critical, it is important that preliminary samples be collected to estimate variability and then to determine the required number of samples.

B.3. Descriptive and Experimental Approaches

Like most ecological field studies, assessment of the effects of contaminants on aquatic ecosystems falls into two broad categories: descriptive and experimental. Descriptive approaches involve routine biomonitoring of either structural and/or functional characteristics of a system. Biomonitoring is one of the oldest and most widely used approaches for assessing the integrity of aquatic systems. Based on the assumptions that 1) the structural and functional integrity of a biological system is a reflection of relative health and 2) that we can define a "healthy" system, numerous studies have attempted to establish

a relationship between species abundance, composition, productivity, etc. and the degree of impact. The distribution and abundance of various groups of aquatic organisms, including protozoans, algae, diatoms, phytoplankton, zooplankton, macroinvertebrates, and fish, have been employed routinely as indicators of the impact of contaminants on aquatic systems.

The concentration of contaminants in aquatic organisms is also frequently employed as an indicator of impact (Prosi 1979). There are several practical reasons for measuring the levels of contaminants in aquatic organisms. Many aquatic organisms bioaccumulate contaminants to levels much greater than those found in overlying water. Since concentrations of contaminants in water are often highly variable and may be below detection, a predictive relationship between contaminants in abiotic samples and organisms may be useful for monitoring impact. Furthermore, since organisms are continuously exposed to contaminants and mobile, they integrate contaminant concentrations over time and space, thus providing a better indicator of contaminant levels in the ecosystem. Moriarity et al. (1984), however, have noted limitations of this approach and suggested that it is often more appropriate to analyze abiotic samples. It is recommended that levels of contaminants be measured in both biotic and abiotic components of aquatic systems at Rocky Flats.

Biomonitoring approaches for evaluating the impact of contaminants typically involve comparison of reference sites to impacted and recovery sites. Ideally, these locations should be similar in all respects except for the presence of contaminants (Green 1979). However, because of natural changes in structural and functional parameters within a system, as well as variation in other parameters such as substrate composition and vegetation, it is often difficult to locate comparable reference and impacted sites. Consequently,

effects caused by the presence of contaminants are confounded by natural changes in aquatic systems. Crossley and LaPoint (1988) note the problems associated with determining changes in complex systems due to spatial and temporal variance, particularly if contaminant effects are subtle. In systems that receive multiple impacts from several sources, such as the systems at Rocky Flats, the determination of specific causes for observed changes is greatly complicated. Green (1979) describes an optimal sampling design for conducting biomonitoring studies, and notes the importance of obtaining pre-and post-impact data from both reference and impacted sites. In this design, spatial and temporal controls are necessary to test the null hypothesis of no change due to impact, where a significant area-by-times interaction indicates significant impact. Hurlbert (1984) criticizes this optimal design and concludes that inferential statistics are not appropriate in most biomonitoring studies because of the problem of temporal and spatial pseudoreplication. This design, argues Hurlbert, allows for the determination of significant differences among locations, but these differences cannot be attributed to a specific cause.

Regardless of the statistical validity of Green's optimal impact, the fact remains that pre-impact data are rarely available in most biomonitoring studies. Consequently, investigators must simply monitor changes in communities during or after impact has occurred and assume a causal relationship.

In the absence of pre-impact data, an alternative to descriptive surveys and routine biomonitoring is experimentation. Experimental approaches involve the use of laboratory toxicity tests, in-situ tests, microcosms, mesocosms, or introduction of contaminants into natural systems. Results obtained from these studies provide the strongest evidence for causal relationships between concentrations of contaminants and community structure or function. Laboratory

and in-situ toxicity tests should be conducted to supplement routine biomonitoring procedures.

C. Field Sampling Procedures

C.1. Physical and Chemical Samples

A variety of physical and chemical variables directly influence the abundance and distribution of aquatic organisms and indirectly affect the responses of these organisms to contaminants. For example, sediment composition, vegetation, depth, and light penetration has a significant effect on abundance of aquatic organisms. The confounding influences of these and other physical/chemical variables must be examined when assessing the impact of contaminants on aquatic systems. Furthermore, numerous physical and chemical factors modify toxicity and bioavailability of contaminants in the field. Toxicity of heavy metals is generally inversely related to water hardness and alkalinity. Both organic and inorganic contaminants readily adsorb to suspended and dissolved materials, thus reducing bioavailability and toxicity. Complexation of contaminants with natural organic materials present in surface water (e.g. ligands) greatly affects bioavailability. Increased temperature and reduced dissolved oxygen concentration generally increase uptake and toxicity of chemicals owing to increased metabolism and gill perfusion. Because of the direct and indirect effects of physical and chemical variables on toxicity and bioavailability, it is important that routine water and sediment quality data be obtained from all sampling locations on each sampling occasion.

C.1.1. Sampling Recommendations

Physical characteristics of all aquatic habitats at Rocky Flats should be evaluated. In lentic systems, important physical characteristics include depth, substrate particle size distribution (i.e. percent clay, fines, sand), organic

content of sediment, and light penetration. In lotic habitats, substrate composition, current velocity, discharge, and slope should also be measured. Temperature, dissolved oxygen, pH, and conductivity should be measured directly in the field at all sites. Water samples (1 L) should be collected from these sites and returned to the laboratory to be analyzed for hardness, alkalinity, and total suspended solids. If phytoplankton and periphyton samples are collected from these sites, water samples should also be analyzed for primary nutrients (ammonia, nitrate/nitrite, and ortho-phosphate). Specific procedures for measuring each of these parameters are described in APHA (1985).

Water samples for contaminant analysis should be collected in acid-washed (for metal analysis) or acetone-washed (for organics analysis) containers. Samples should be immediately placed on ice and transported to the laboratory. Sample preparation and analytical techniques will depend on the class of contaminants being measured. Analysis of metals should be conducted using flameless atomic absorption spectrophotemtry. Organic contaminants should be analyzed using either gas chromatography (GC) or high pressure liquid chromatography (HPLC).

C.2. Phytoplankton and Zooplankton

Planktonic communities of lentic habitats consist of a diverse group of microscopic plants (phytoplankton) and animals (zooplankton) floating, drifting, or feebly swimming in the water column. The study of these communities, particularly the feeding relationships between phytoplankton and grazing zooplankton, has been one of the more productive areas of research in aquatic ecology.

In terms of biomass, the chlorophyll-bearing blue-green algae (Cyanophyta) and green algae (Chlorophyta) are usually the dominant components of lentic

systems. These organisms form the base of the food chain in open lentic systems and therefore may be responsible for the transport of contaminants to grazing zooplankton and fish.

C.2.1. Sampling Recommendations

C.2.1.1. Phytoplankton

A variety of sampling devices are available for estimating phytoplankton abundance and species composition. Two general types of sampling devices most commonly employed in lentic habitats are closing samplers and net samplers. Net samplers are lowered to a specified depth and retrieved through the water column, thus collecting a composite sample. Mesh size should be no larger than 80 μm. Total volume of water sampled should be estimated using a flowmeter attached to the net. Although net samplers are useful for qualitative analysis of phytoplankton, they are not recommended for quantitative estimates since some organisms may pass through the mesh. Closing samplers (e.g. the Kemmerer, Juday, and Van Dorn types) collect a known volume of water from a specified depth and are therefore useful for quantitative evaluation of plankton abundance (number per m^3). Necessary sample volume will depend on productivity and density of organisms. In the shallow, productive ponds located at Rocky Flats, it is recommended that at least 1 L of water be collected per replicate for analysis of phytoplankton abundance and composition. Samples should be preserved in 5% buffered formalin. For enumeration and identification of phytoplankton, samples should be concentrated using sedimentation, centrifugation, or filtration. Quantitative analysis of phytoplankton requires a high quality compound microscope for species identification. Abundance is expressed as number per unit volume and therefore the ocular must be equipped with a Whipple grid micrometer for calibration. At high phytoplankton density, the samples should be diluted.

Concentrations of phytoplankton should be determined using a Sedgwick-Rafter counting chamber.

C.2.1.2. Zooplankton

Quantitative analysis of zooplankton composition and abundance may be obtained using either closing samplers or net samplers. Because of a lower abundance of zooplankton compared to phytoplankton, larger samples may be necessary and therefore vertical or horizontal tows that sample at least 10 L of water are recommended. Since ponds located at Rocky Flats are relatively shallow, vertical stratification of zooplankton is not a serious problem and thus collecting replicate samples from several depths will not be necessary. Zooplankton samples should be preserved in 5% buffered formalin. In the laboratory, zooplankton samples should be concentrated using either sedimentation or filtration through a net of similar mesh size as that used during collection.

Quantitative analysis of zooplankton is accomplished using a calibrated microscope and counting chamber for smaller organisms (rotifers, nauplii) and a dissecting scope for adult zooplankton. All counts should be expressed as numbers per unit volume.

C.3. Periphyton and Algae

Periphyton communities consist of a diverse assemblage of organisms attached to underwater surfaces. Aquatic microbes, fungi protozoans, algae, and diatoms are the most common groups comprising the periphyton; however, most biomonitoring studies have focused on algae and periphyton. Algae and periphyton are important components of both lentic and lotic systems and are the principal primary producers in many systems. These organisms form the base of aquatic food chains and therefore are important in the transfer of energy and contaminants to higher trophic levels. More importantly, these groups are highly sensitive to

contaminants and therefore are good indicators of water quality (Patrick 1957; Cairns et al. 1971). Much of the early research in pollution biology focused on the distribution and abundance of these organisms (Carpenter 1924; 1925).

Changes in species composition of algae and periphyton are the most commonly employed indicators of water quality. Because of considerable variation in response to contaminants among taxa, species composition will often differ dramatically at impacted and recovery sites. Other structural indices commonly employed in the analysis of these organisms include total number of species, species diversity, biomass, and community similarity.

The use of algae and periphyton as indicators of water quality may be limited due to taxonomic difficulties. For example, Patrick (1978) notes that a typical diatom community in an unpolluted habitat may consist of 300-400 species. If the taxonomic expertise required to identify these organisms is available, then analysis of periphyton communities is an excellent indicator of water quality.

C.3.1. Sampling Recommendations

Periphyton communities should be collected at least seasonally from all surface water sites (A, B, and C series ponds, Lindsey pond, and all streams) on either natural or artificial substrates. Qualitative samples should be collected from natural substrates by scraping the surface of submerged materials. A volume of 5-10 mL collected from a variety of different substrates is sufficient. Since the greatest abundance and diversity of periphyton is found in shallow littoral areas of ponds and riffles of streams, qualitative sampling should be limited to these areas.

Quantitative analysis (#/cm^2) of periphyton on natural substrate is possible if samples are collected from a measured area; however, these samples

are often highly variable. As a result, many samples may be required to detect significant differences among locations.

A more rigorous method for obtaining quantitative periphyton samples involves the use of artificial substrates. Most commonly, samples are collected on glass slides placed in a plexiglass rack and colonized for a period of 3-4 weeks (see Gale et al. 1979 for a description of diatometers). The necessary period of colonization will vary depending on season and location. Initial periphyton growth (1-2 weeks) on clean substrates is exponential and then declines. Thus optimal sampling is usually after 3-4 weeks of colonization (EPA 1973). One of the disadvantages of the above approach is that artificial substrates are often selective for certain taxa. As a result, relative abundance of species on artificial substrates may not be an accurate reflection of abundance on natural substrates. Since the problem of sampling selectivity will be similar among locations, this may not be a severe limitation. More importantly, this problem is clearly offset by the advantages of reduced variability and greater precision associated with using standardized substrates of similar material and size.

The number of artificial substrate samplers required will depend on sampling variability and desired precision (see above). Additional samplers should be placed in all ponds and streams to account for loss of samplers.

Glass slides should be scraped with a razor blade and samples should be preserved in 5% formalin. In the laboratory, algae should be enumerated using a Palmer-Maloney plankton counting chamber under 400X total magnification. Counting should proceed by making a single pass through the long axis of the chamber and stopping when the 500th organism is counted. If less than 500

organisms are counted, then another sample should be prepared and enumerated. Dense samples should be diluted.

In addition to enumeration of algal density, biovolume of dominant taxa should also be estimated. Biovolume should be estimated using mensuration formulas that approximate the geometric shape of each taxon (Beyer 1981). The product of biovolume and density allows easy comparison of dominant taxa and is a more realistic indicator of community structure than abundance.

C.4. Biomass and Productivity

Total biomass of phytoplankton, periphyton, and zooplankton should be estimated using dry weight and ash-free dry weight of samples collected from each pond. Dry weight is determined by placing a known volume of concentrated sample in a pre-weighed ceramic crucible and drying at 100 °C for 24 h. Ash-free dry weight of plankton and periphyton is determined by placing these dried samples in a muffle furnace at 500 °C for one hour. The amount of organic material present in a sample is calculated by subtracting the ash-free dry weight from the dry weight.

Biomass of phytoplankton and periphyton should also be estimated by measuring chlorophyll *a*. This pigment is extracted with acetone and the concentration is measured using either trichromatic or fluorometric methods. Concentrations in the sample are expressed as μg/L.

Phytoplankton and periphyton productivity should be measured using the ^{14}C method, which allows for the calculation of total carbon assimilation by these primary producers. Methods for the determination of biomass and productivity may be found in APHA (1985), Sladecek and Sladeckova (1964), and Crossey and LaPoint (1988).

C.5. Benthic Macroinvertebrates

The distribution and abundance of benthic macroinvertebrates are routinely employed as indicators of water quality. Because of their influence on various functional parameters in aquatic systems, such as primary productivity, detritus processing, and energy flow, benthic macroinvertebrates are an important component of aquatic habitats. These organisms are often quite abundant, have a relatively short generation time, and represent several functional feeding groups. Finally, because of their close association with the substrate, tendency to bioaccumulate toxic materials, and their importance in aquatic food chains, benthic invertebrates are useful for monitoring the transport of contaminants in aquatic systems.

Considerable research effort has been devoted to describing responses of benthic communities to contaminants. To be useful as an indicators of contaminant impact, these responses should be predictable, allowing some degree of generalization among locations. Winner et al. (1980) reported that responses of benthic invertebrates to pollution are predictable and proposed using benthic community structure as an index of heavy metal pollution. In a study of the relationship among metal concentrations, water quality criteria, and benthic community structure in 15 U.S. streams, LaPoint et al. (1984) noted that benthic communities responded in a "predictable and indicative manner, which overall may be more sensitive than any single species [toxicity] tests." Clements et al. (1988) compared community responses of benthic invertebrates to contaminants in the field and in outdoor experimental streams and concluded that these responses were highly predictable.

C.5.1. Community Indices

Several indices of community structure have been employed to assess the impact of contaminants on benthic organisms. The most common indices include total abundance (number per m^2), number of species per sample, and species diversity. Winner et al. (1975) compared the sensitivity of several community indices (abundance, number of species, Margalef Index, Shannon Diversity) to contaminants and concluded that the number of species was the most sensitive index examined. Although total macroinvertebrate abundance is also a sensitive indicator of stress, it is much more variable, thus making it difficult to distinguish between impacted and reference areas.

Change in percent composition of dominant macroinvertebrate taxa is probably the most useful indicator of the impact of contaminants. Because benthic organisms show considerable variability in their sensitivity to toxicants, differences in percent composition among field sites may be employed to assess the degree of contamination. This approach requires that appropriate reference sites be available for comparison.

In summary, the distribution and abundance of benthic invertebrates have been employed extensively in both descriptive and experimental studies for documenting the impact of contaminants on aquatic systems. Responses of these communities are highly sensitive and therefore useful indicators of water quality. It is recommended that these organisms be included in both descriptive (biomonitoring) and experimental (laboratory toxicity tests) investigations at Rocky Flats.

C.5.2. Sampling Recommendations

Benthic macroinvertebrates should be collected at least quarterly from both lentic and lotic habitats at Rocky Flats. Replicate samples should be collected

from A, B, and C Series Ponds in addition to Lindsey Pond (reference). Samples should be collected using both artificial substrates (Hester-Dendy multiplate samplers) and a Ponar grab. Hester-Dendy samplers should be suspended in the water column above the substrate and allowed to colonize for at least 30 days. The substrates should be retrieved using a fine mesh net to prevent the loss of organisms. Because of their uniform size and composition, artificial substrates have the advantage of greater precision and reduced sampling variability. Thus, fewer samples are necessary to detect differences between control and contaminated sites. However, since these devices are usually selective for certain taxa, quantitative analysis of benthic community structure also requires direct sampling of the substrate. Stream samples should be collected from both on-site and off-site locations in North Walnut Creek, Rock Creek, and Woman Creek. For reasons described above, samples should also be collected using both artificial substrates (rock-filled trays or baskets) and quantitative (e.g. 0.1 m^2 Surber sampler, Hess sampler) methods.

All benthic samples should be washed through a 500 μm mesh sieve in the field and the organisms retained should be preserved in 10% formalin. Staining the samples with a small amount of rose bengal prior to preservation facilitates sorting. Samples should be sorted in white enamel pans under 10 X magnification. All organisms should be identified to the lowest practical taxonomic level (usually genus or species). Good general taxonomic keys that will allow identification of most benthic macroinvertebrates to the level of genus are Merritt and Cummins (1984) and Pennak (1978). Identification of dominant taxa to species may require consultation with appropriate experts.

C.6. Bioconcentration of Contaminants by Benthic Macroinvertebrates

Because of their close association with sediments and the ability of certain species to tolerate high concentrations of sediment contaminants, benthic macroinvertebrates rapidly bioaccumulate organic and inorganic chemicals either directly from sediments or from interstitial water. For reasons described above, concentrations of chemicals in benthic organisms are better indicators of the presence of contaminants than levels in abiotic samples. First, concentrations in these organisms may be several orders of magnitude greater than in overlying water. Due to limitations of analytical techniques at low concentrations and high variability associated with levels of contaminants in water, levels in benthic macroinvertebrates are easier to measure. Second, since benthic invertebrates are continuously exposed to contaminants, they integrate the effects of these contaminants over time. Finally, because of their importance in aquatic food chains, benthic invertebrates may be an important source of contaminants to higher trophic levels.

C.6.1. Sampling Recommendations

Decisions regarding which species to include in these analyses should be based on both numerical abundance and ecological relevance. Quantitative analysis of contaminants in benthic invertebrates will require obtaining sufficient biomass of material. In addition, replicate samples should be collected from each location. Also, since different extraction and analytical procedures will be required for each class of contaminants, (metals, organics, radioactive materials) separate samples must be collected for each class. Thus, collections should be restricted to taxa that are abundant and available throughout the year. With respect to ecological relevance, analysis of contaminants in benthic organisms should include taxa that are important in the

diet of fish and waterfowl and thus most likely to represent a source of contaminants to these predators. Preliminary sampling should be conducted to determine seasonal abundance of benthic macroinvertebrates and their relative importance in the diets of these predators.

Benthic macroinvertebrates should be collected from all aquatic habitats at Rocky Flats using grab samplers or sweep nets. Dominant taxa should be sorted and identified in the field and placed in acid-washed (metals analysis) or acetone-rinsed (organics analysis) vials. In the laboratory, dry weights of these organisms should be determined (see above) and tissue samples should be ground and extracted using procedures appropriate for the particular contaminants of concern. Concentrations of contaminants in benthic macroinvertebrates should be expressed in units of $\mu g/g$.

C.7. Fish

Sampling resident fish populations is an important component of any ecological assessment of impact. Because many species of fish occupy the upper trophic levels of aquatic food webs, they may be either directly or indirectly affected by the presence of contaminants. Various ecologically relevant and highly sensitive endpoints may be measured in the field, including survivorship, growth, reproduction, relative and absolute density, condition, and species richness. Considerable research effort has been devoted to assessing biological integrity of aquatic systems based on community structure of fish populations (Karr 1981).

C.7.1. Sampling Recommendations

We recommend that fish be sampled from all ponds and streams at Rocky Flats for the primary purpose of measuring levels of contaminants present in these organisms. Fish may be sampled by electrofishing or by the use of seines.

Specimens should be immediately placed on ice and transported to the laboratory. All individuals should be identified to species, measured, weighed, aged, and sexed. Muscle, liver, kidney, brain, and gonadal tissue should be dissected and analyzed for the presence of contaminants. Tissue samples should be carefully dissected to avoid cross contamination. Tissue dry weights should be determined and samples should be ground and extracted using appropriate procedures. Concentrations of lipophilic contaminants in tissue samples are highly dependent on percent lipids content. Thus all tissue analyses should be corrected for percent lipids so that comparisons among species, size classes, ages, sexes, and tissue types will be meaningful.

D. Toxicity Testing

Although field sampling may provide information on the presence of contaminants in a particular habitat, this approach provides little insight into the potential toxicity of these contaminants. The presence or absence of organisms in an area is a complex function of physical, chemical, and biological characteristics of the system. As a consequence, it is often difficult to determine direct cause and effect relationships between the presence of contaminants and community structure or function. Furthermore, results of field biomonitoring often show all-or-none responses and may provide little indication of the relative degree of contamination. For these reasons, as well as those described above (see Descriptive and Experimental Approaches), experimental approaches are recommended to supplement field biomonitoring.

D.1. Laboratory and <u>In-Situ</u> Toxicity Tests

Toxicity tests are routinely employed to estimate the potential adverse effects of contaminants on aquatic organisms. Typically these tests employ surrogate species and require that investigators assume responses of these simple

laboratory procedures are indicative of responses in more complex systems (i.e. ecosystems). Although these tests have been criticized because of their lack of environmental realism (Cairns 1983), when used in conjunction with field monitoring they provide necessary supporting evidence for determining the degree of impact of contaminants.

Common endpoints employed in laboratory toxicity testing include mortality, growth, reproduction, and developmental and behavioral abnormalities. Acute toxicity tests measure mortality and usually involve short-term exposure (48-96 h). More sensitive chronic and early life stage tests involve long-term exposures at much lower concentrations. Freshwater organisms most frequently employed in these tests include cladocerans (Daphnia magna and Ceriodaphnia dubia) and the fish (Pimephales promelas, Salmo gairdneri, and Lepomis macrochirus).

Numerous potential modifying factors exist that influence toxicity and bioavailability of contaminants in the field (see Physical and Chemical Samples). Since many of these factors cannot be easily controlled or manipulated in the laboratory, *in-situ* toxicity tests are recommended to account for the influence of these factors. *In-situ* experiments generally involve placing test organisms in open chambers at reference and impacted sites in the field. Similar endpoints as described above may be examined and results should be compared to those obtained in laboratory experiments.

Sediments are an important sink for contaminants in aquatic systems. Concentrations of organic and inorganic pollutants in sediments are often several orders of magnitude greater than in overlying water. Due to various physical and biological processes, sediment contaminants may be released and made available to aquatic organisms. The effects of contaminated sediments on aquatic organisms

should also be examined in the laboratory and results obtained should be compared to results of field studies. The recommended approach is known as the "sediment quality triad" (Chapman 1986). Briefly, the approach involves chemical characterization of contaminated sediments, analysis of the distribution and abundance of benthic organisms within the sediments, and sediment toxicity tests. Results obtained provide information on the degree of sediment contamination and the potential effects of these contaminants on aquatic organisms.

Numerous documents are available describing specific methods for conducting acute and chronic toxicity tests (EPA 1975; ASTM 1980; ASTM 1981). A good general description of acute, chronic, and early life stage testing procedures is provided by Rand and Petrocelli (1985). Methods for evaluating effects of sediment contaminants on aquatic organisms are currently being developed by the American Society for Testing and Materials. Draft copies of these documents are available from ASTM (see Nelson et al., in review).

D.1.1. Testing Recommendations

D.1.1.1. Surface Water

Acute and chronic toxicity tests should be conducted with at least two surrogate species. Because of the amount of background data available on the sensitivity of Ceriodaphnia dubia and Pimephales promelas to contaminants, these organisms are recommended for testing. In addition, at least one indigenous species, preferably a species collected from reference sites at Rocky Flats, should be employed. Toxicity tests should be conducted with water collected from all ponds and streams located at Rocky Flats. Appropriate reference sites for the A, B, and C series ponds as well as upstream reference areas for lotic systems should be located. To improve the statistical reliability of these data, replicates of each treatment concentration should be tested. Toxicity tests

should be conducted at least seasonally to account for seasonal variation in acute and chronic effects. Survivorship, growth, reproduction, etc. of test organisms in water collected from contaminated and reference sites should be compared. The suitability of potential reference sites at Rocky Flats should be evaluated by comparing these same endpoints in water from reference sites to laboratory water. Toxicity tests should also be conducted using reference toxicants to evaluate the health of all test species.

Standard water quality variables (e.g. temperature, dissolved oxygen, pH, hardness, alkalinity, conductivity) should be collected routinely during these toxicity tests. In addition, concentrations of contaminants should be measured in each replicate container.

D.1.1.2. Sediments

Since sediments are most likely an important sink for contaminants at Rocky Flats, sediment toxicity tests should be conducted. As noted above, specific methods for conducting solid phase and elutriate toxicity tests are currently being developed. Briefly, these tests involve exposing organisms to either whole sediments collected from contaminated sites (solid phase tests) or pore water extracted from these sediments (elutriate tests). Recommended benthic organisms for whole sediment tests include chironomids (Chironomus riparius, C. tentans), amphipods (Hyalella azteca), and mayflies (Hexagenia limbata). Nonbenthic organisms used in elutriate tests include Ceriodaphnia dubia and Daphnia magna). To obtain necessary dilutions, contaminated sediments may be mixed with clean sediments. Alternatively, elutriates obtained from contaminated and clean sediments may be mixed. Because of the complications associated with sediment dilution, Giesy et al. (1990) recommended that tests be conducted with pore water. Since experiments with pore water may be conducted with standard

(nonbenthic) test organisms (e.g. Ceriodaphnia dubia), results may be compared to the wealth of data available from surface water tests.

Sediment toxicity tests should be conducted using sediments collected from the A, B, and C series ponds at Rocky Flats. These sediments should be collected from the same locations where benthic samples were collected. Ideally, benthic samples collected with a ponar (see above) should be split into three subsamples: one for chemical analysis, one for analysis of benthic communities, and the final portion for sediment toxicity tests. Reference sediments should be collected from Lindsey Pond. Sediments should be returned to the laboratory and pore water extracted using either filtration and/or centrifugation techniques. Appropriate dilutions with reference sediment extracts should be obtained. Tests should be conducted with benthic (C. riparius) and nonbenthic (C. dubia) organisms using techniques employed in standard toxicity tests. Recommended endpoints include mortality, growth, and emergence for C. riparius and mortality and reproduction for C. dubia.

D.2. Bioconcentration of Contaminants from Water and Sediment

To investigate the potential transfer of contaminants at Rocky Flats, bioconcentration of contaminants by aquatic organisms should also be investigated in the laboratory. Recommended test organisms include the fathead minnow for water exposures and the chironomid, Chironomus riparius, for sediment exposures. Organisms should be exposed to contaminated water or sediments in the laboratory and concentrations of contaminants in tissue samples should be measured on several occasions. Estimates of uptake rate, depuration rate, and equilibrium tissue concentrations obtained from these experiments will provide information on the potential transfer of contaminants within aquatic systems at Rocky Flats.

Bioconcentration of contaminants measured experimentally in the laboratory should be compared to data obtained from the field.

E. Summary and Conclusions

The approach recommended for evaluating surface water quality at Rocky Flats will require highly integrated laboratory and field investigations. Since it is unlikely that any one group will have the technical expertise for all phases of this evaluation, an interdisciplinary approach involving several participating groups will be necessary. This will require considerable coordination among the various groups in order to avoid duplication of effort. More importantly, coordination of laboratory experiments, chemical analyses, and field biomonitoring is essential to validate results of each phase of this research. For example, the ability of laboratory toxicity tests to demonstrate direct effects of contaminants will be greatly improved if conducted in conjunction with the field monitoring approach described above. Similarly, due to limitations of routine field biomonitoring, laboratory experiments are necessary to document the relative degree of contamination and the potential for food chain transport of contaminants. Therefore it is strongly recommended that laboratory toxicity tests and field biomonitoring be conducted simultaneously. Water and sediments for toxicity tests should be collected from the same locations where chemical and biological samples are obtained. Results obtained from such a coordinated effort will be necessary for assessing the relative degree of contamination of aquatic systems at Rocky Flats and thus establishing priorities for remediation.

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V. ASSESSMENT OF RADIATION IMPACTS ON TERRESTRIAL AND AQUATIC ORGANISMS

(by F. W. Whicker, Department of Radiology and Radiation Biology)

A. Introduction

The effects of ionizing radiation on organisms can be examined at various levels in the biological hierarchy. The appropriate level at which to examine effects on non-human organisms is the population. The primary radiation-induced impacts that are manifest in population level changes are reproduction and survival. In almost all cases, reproduction can be impaired at lower dose rates than can survival, so reproduction is the appropriate and most sensitive endpoint for assessing ecological impacts of ionizing radiation (IAEA 1988).

The primary, fundamental predictor of the possibility of radiation induced impacts at the population level is the radiation absorbed dose rate (measured in rad or Grey per unit time) and the type and energy of the radiations involved (e.g. alpha, beta, and gamma radiation). If the total dose and dose rate to specific biological tissues can be estimated, it is possible to use dose-response relationships from published scientific works to predict effects on individual organisms, as well as on the exposed population. It is usually not feasible to directly measure dose to tissues of most organisms. A more reasonable approach is to measure the concentrations of radionuclides in various tissues and environmental compartments. Using this information, as well as data on geometrical relationships, energies and types of radiation, etc., it is possible to calculate the absorbed radiation dose to critical tissues. The critical tissues from the standpoint of reproduction are the reproductive organs of plants and animals.

Therefore, the task of assessing the potential ecological impacts of radionuclides in the environment is based first upon measurements of radionuclide

concentrations in key tissues and environmental media (the latter of which gives rise to external radiation exposure). Next, radiation absorbed doses and dose rates are estimated. Finally, the dose estimates are entered into the appropriate dose-response relationships to predict the nature and magnitude of any impact. Depending on the extent of the contaminated area, and the duration and magnitude of the contamination, actual radiation impacts on the population may or may not be observable in the exposed populations.

In this section, a general guide for evaluating ecological impacts of radioactive materials is presented. The guide is more philosophical than prescriptive, but enough detail is given to provide a methodological framework in which to work.

B. Measurement of Radionuclide Concentrations

B.1. Types of Samples to be Taken

In terrestrial ecosystems, the primary components that need to be sampled include soil (at various depth increments), litter, vegetation (particularly the reproductive and meristematic tissues), and animal tissues. Vegetation can be sampled in broad categories, such as grasses, forbs, shrubs, and trees, since radiosensitivity can be generally predicted on the basis of life-form (Whicker and Fraley 1974). Animal sampling should be restricted to those species with home ranges expected to be as small or smaller than the size of the contaminated area and those that are comparatively sensitive to radiation. As a rule, small mammals are good candidates for study. Tissues such as bone, liver, muscle, lung, and reproductive organs should be considered for analysis.

In aquatic ecosystems, water, sediment, macrophytes, fish, and other vertebrates such as turtles should be sampled. Water samples should be divided into seston and filtered water. Sediment samples should be stratified into

≈ 1 cm depth increments. Vertebrate tissues to be assayed should include skin, bone, muscle, liver and reproductive organs (and/or eggs if present).

B.2. Sampling Design

The contaminated area to which statistical inferences will be made should be spatially defined. This should be the entire SWMU, or portions thereof if it is heterogenous. If spread of contaminants to adjacent areas is possible, such areas should be included in the design. A sufficient number of replicate samples should be taken from randomly-chosen locations to permit reasonable estimates of means and variances. A comparable number of samples from carefully-chosen, uncontaminated reference sites should be taken to establish "background" levels of contaminants. Reference sites should be ecologically similar to the SWMUs, but not contaminated by Plant operations. Preliminary sampling or review of published literature (e.g. Little et al. 1980) will be required to estimate sampling variance, which must be known before an adequate sample size can be determined (see Sections II.E.2.1. and IV.B.2.). Ecosystem compartments expected to undergo significant seasonal fluctuations should be sampled several times throughout the calendar year. This would likely include water and macrophytes in aquatic systems; and vegetation and small mammals in terrestrial environments. A single sampling time is likely sufficient for long-lived radionuclides in sediments and soils, provided that physical disturbance of the area is not occurring and that on-going dispersal of contamination is non-existent or very minor.

B.3. Sampling and Sample Preparation

The concentrations of the radionuclides expected to occur in the environment at Rocky Flats will vary widely from media to media and from sample to sample. This is particularly true for soil and sediment samples, which

exhibit great spatial variability and which, in general, contain much higher radionuclide concentrations than water or biological tissues (Little et al. 1980). This necessitates extraordinary care to prevent cross-contamination between samples. A few grains of soil contaminating a tissue sample, for instance, can easily contribute enough radioactivity to completely invalidate the analytical result for the tissue.

Great care should be exercised in the field to prevent contact between soils, sediments, and other samples (Klement 1982). Soil and sediment layers should be carefully separated in such a manner that minimizes contact or mixing. Separate vegetation and animal specimens should not be allowed to contact other samples. Animal specimens should have unbroken and completely intact surfaces to prevent dust from contacting internal parts (this is achieved in the case of small mammals by using live rather than snap traps). Sampling tools should be cleaned between samples and sampling sessions. All samples must be at least doubly bagged with carefully sealed plastic bags or other dust-tight containers. Bagged specimens should be placed in clean, heavy-duty coolers or other containers to assure safe transport to the laboratory.

In the laboratory, specimens should be processed in appropriate hoods in batches sorted on the basis of sample type and expected concentrations of radioactivity. Hood surfaces, tools, sieves, etc. should all be carefully cleaned between individual samples or sample batches, as appropriate. Dissection of animal tissues is best carried out in a lominmar flow bench in which air contacting the operation has been filtered through a HEPA (high efficiency particulate) filter. Laboratory spaces should be segregated so that samples with the higher potential or expected concentrations of radioactivity (soils and sediments) are processed in areas separate from where samples with much lower

expected concentrations are to be processed. Soils and sediments must be sieved before analysis for radionuclides takes place (Little and Whicker 1978). A 2 mm diameter mechanical sieve should first be used to remove stones, twigs, and other extraneous material. Material < 2 mm can be assayed without further sieving to provide data for an initial evaluation. Unless the samples contain quite high concentrations of radioactivity (> 1 nCi/g) there is little need to get into further (~ 50 g/sample) should be sampled and prepared for analysis to allow archiving for reanalysis should this become necessary.

Vegetation from terrestrial plots should be divided into two fractions for processing. One fraction should be dried then ground as is for analysis. The other fraction should be ultrasonically cleaned to remove surficial dust prior to analysis. The method outlined in Skinner (1982) is recommended. This separation will permit a more accurate assessment of the location of radioactivity in plant tissue, and thus a better estimate of dose and possible impact (Arthur and Alldredge 1982).

B.4. Radionuclides to be Measured

The primary mission of Rocky Flats necessitates the handling of large quantities of plutonium. Therefore, the radioactive materials that have reached the environs of the Plant site from routine and accidental releases are dominated by isotopes of plutonium and by radioactive products of plutonium decay.

The plutonium isotope of primary concern at Rocky Flats is ^{239}Pu, which primarily emits alpha particles and has a 24,000 year half life. Plutonium-238, ^{240}Pu, ^{241}Pu, and ^{242}Pu are other isotopes of plutonium that can be measured at some sites (USDOE, 1980). Plutonium-241 is abundant on an activity basis, but it is a weak beta emitter with a 13 year half life and is not considered as hazardous as ^{239}Pu or ^{240}Pu. However, ^{241}Pu decays to 458 year (half life) ^{241}Am,

which emits energetic alpha particles, as well as 60 kev photons. The photon emissions of ^{241}Am, makes it easy to detect with portable instruments in the field, as well as making this isotope a potential external radiation hazard. It may be calculated that ^{241}Am will continue to increase in activity from historical deposits of ^{241}Pu, reaching a peak on or about the year 2033 and slowly decline thereafter (Krey et al., 1976).

Other historical releases of radionuclides to the environment at Rocky Flats include uranium and tritium (USDOE, 1980). Above-background levels of uranium have been observed in pond sediments on the Plant site and tritium releases to aquatic ecosystems have also been documented. Should a criticality accident ever occur at Rocky Flats, there would be the possibility of fission product (e.g. ^{131}I, ^{137}Cs, ^{90}Sr, etc.) releases, but such releases have not been reported at Rocky Flats.

In view of the nature and magnitude of the radioactive components that might be expected in Rocky Flats wastes and effluents, a stepwise approach to the evaluation of potential radiological impacts is recommended. The first step in the field should be an external survey with portable gamma, beta, and alpha survey meters to evaluate the safety of the sampling process. Once samples are taken, they should be dried by a procedure that captures sample moisture. This moisture should be assayed for tritium using liquid scintillation counting. If the tritium exceeds drinking water standards (20 Nci/L), this radionuclide should be carefully evaluated. Because of the high expense of doing a radiochemical analysis for specific isotopes of plutonium and uranium, a gamma ray spectral analysis of all samples is recommended as the next step. This assay can be done on bulk samples, it is rapid, and relatively inexpensive. The quantities of ^{241}Am and certain isotopes of uranium, thorium and most fission products can be

easily measured by gamma spectrometry. The presence and amount of ^{241}Am is usually a good indicator of plutonium isotopes as well.

The next step should be a gross alpha and gross beta analysis of strong acid extracts of all samples (Harley 1970). This procedure is also relatively inexpensive, but it will reveal the presence of alpha and beta emitting radionuclides, especially if they are sufficiently concentrated to be of concern from the standpoint of ecological risk. If the concentrations of gross alpha or beta radioactivity are less than about 1 nCi/g in biological tissue (37 Bq/g or 37 dio/s-g), or less than about 10 nCi/g (370 Bq/g) in soil or sediment samples, there is little justification for going to the expense of an isotopic analysis. At these levels, the dose rates to organisms are likely to be less than 1 rad/day (10 mGy/day), which are highly unlikely to produce measurable perturbations in most populations (IAEA 1988).

If the gamma ray spectral analysis and/or the gross alpha or beta analysis indicated the possibility of high isotopic concentrations (> 1 nCi/g for biological tissues; > 10 nCi/g for soils and sediments), a more complete assessment is indicated. This would include a radiochemical determination, using alpha spectrometry, of $^{239,240}Pu$, ^{238}Pu, and isotopes of uranium. If high concentrations of fission products were detected by gamma rap spectrometry, a radiochemical analysis for ^{90}Sr should be conducted.

B.5. Methods for Radionuclide Analysis

It is probable that field sampling procedures will require the assistance of Rocky Flats radiological monitoring personnel to assure the safety of the workers, as well as the potential hazard of transporting samples. Routine gross alpha monitors are normally employed when plutonium contamination is a possibility.

The use of a monitoring device such as a FIDLER (Krey et al. 1976) which effectively measures photon emissions from ^{241}Am is also recommended. If the presence of fission products is suspected, a portable Geiger-Mueller detector and rate meter should be used to monitor samples and workers in the field.

The collection of moisture from field samples for tritium analysis is usually done using a freeze-dry apparatus with cold traps for water collection. This water can be added to approprial cocktails in vials designed to be accepted by a liquid scintillation counter. These methods are simple and well-standardized (Vose 1980; Hartley 1970).

Scatting of bulk samples for the presence and quantity of gamma emitting radionuclides should be done with state-of-the-art germanium detectors and multichannel analyzers (Klement 1982). Large germanium detectors that offer very high spectral resolution, as well as good sensitivity, are currently available. The resolution of gamma energies is so good with modern systems that simultaneous, unambiguous identification and quantification of many radionuclides in a single sample is routine. Samples should be prepared to achieve uniform geometrical configurations and sample densities. Standards prepared in identical configurations and densities and spiked with known quantities of reference radionuclides should be used to determine counting yields.

Radiochemical determinations for alpha emitting radionuclides should be carried out only by qualified laboratories that use accepted techniques (e.g. Sill and Williams 1969) and through QA/QC procedures. These determinations typically involve sample dissolution or extraction, chemical isolation of the radionuclides desired, electroplating, and alpha spectrometry. Isotopic tracers should be used to evaluate chemical recoveries of the radionuclides. Similar steps for pure beta emitting radionuclides can also be expected.

It is imperative that good QA/QC procedures are followed throughout (Klement 1982). These include, but are not limited to, the use of blanks, duplicates, spiked unknowns, and independent analyses by high quality laboratories to assure the reliability of the analytical results.

C. Calculation of Dose Rates

Concentrations of radionuclides in biological tissues crucial for reproduction (gonads in animals; flowering parts and meristems in the case of higher plants), and in soil, sediment, water and air can be used to estimate the radiation absorbed dose rate in critical tissues. The absorbed dose rate, measured in rad or Gy per unit time, is a measure of the energy absorbed per unit time by a given mass of tissue. The energy deposition results from internally incorporated radioactivity as well as external radiation from surrounding tissues, soil, water, etc. The rad is defined as 100 erg/g and the Grey (Gy) as 1 J/kg; thus 1 Gy = 100 rad.

The basic method to calculate dose rate from incorporated radioactivity is to multiply the tissue concentration (in disintegrations/g time) by the absorbed energy per disintegration (e.g. Joules/disintegration). The value of the second term depends on the type and energy of the radiation, as well as the geometrical configuration of the tissue. The dose from external radiation also depends on the type and energy of the radiations, geometrical relationships of the target tissue to the surrounding media, and concentrations of radionuclides in surrounding media. Neither internal nor external dose rates are simple and straightforward to calculate; however, simplifying assumptions can be made to get upper estimates of dose rate (IAEA 1988). Helpful reference for estimating internal and external dose include Till an Meyer (1983), IAEA (1982), Whicker and

Schultz (1982) and IAEA (1988). It is highly recommended that a specialist in radiation dosimetry be consulted to assist with calculations of dose.

D. Evaluation of Data

Once dose rate estimates to critical biological tissues have been made, it is possible to estimate the potential for population-level perturbations arising from radionuclide contamination. The best single reference for making this determination is IAEA (1988). This document includes a thorough literature review on the effects of acute and chronic radiation on populations of plants and animals in both aquatic and terrestrial environments. A general conclusion is that dose rates less than 1 mGy/day to critical tissues are not likely to cause observable changes in biological populations. In fact in most instances, dose rates less than 10 mGy/day are not likely to be deleterious to the environment. Other data on radiation effects, specifically pertinent to Rocky Flats, should be reviewed. This includes an article on searches for radiation impacts in terrestrial plant and animal populations near the 903 Pad (Whicker 1980).

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